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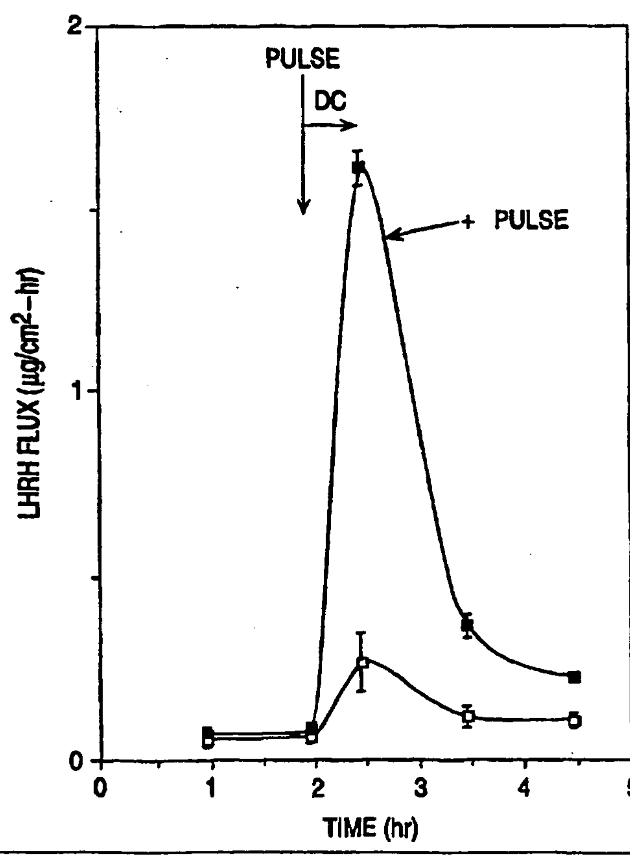
(57) Abstract

The invention relates to a method for pulsed transport of a substance through tissue, the method comprising the steps of: (a) applying at least one electrical pulse to the tissue causes electroporation of the tissue region, the electrical pulse being applied for an electroporation pulse duration, the electroporation pulse duration being shorter than the driving force duration; (b) applying a driving force to the region of tissue whereby the driving force causes the substance to be transported through the tissue for a driving force duration; and (c) repeating step (a) during the driving force duration.



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PULSATILE DELIVERY SYSTEMS OF BIOLOGICALLY ACTIVE AGENTS USING ELECTRO VOLTAGE PULSING FOR CONTROLLING MEMBRANE PERMEABILITY

FIELD OF THE INVENTION

The present invention relates to transdermal delivery of biologically active agents and other substances in a controlled manner.

BACKGROUND ART

Constant drug delivery with steady state rates has been used in many controlled release devices such as transdermal patches and oral tablets (osmotic pump). Iontophoresis has been used for delivery of substances across tissues. The rate of substance delivery, however, is limited by the iontophoretic mechanism, such as the nature of the substance, the nature of the delivery site on the tissue, the structure and composition of the substance reservoir, etc. The ability to control the iontophoretic delivery rate to transfer increased levels of the substance for short periods of time on demand or according to a preset schedule is limited.

Under many circumstances, pulsatile drug delivery with variable delivery rates during the therapy treatment offers better therapeutic advantages. Many mechanisms have been studied to generate pulsatile types of delivery profiles, including iontophoresis, magnetically modulated drug delivery systems, temperature responsive controlled drug delivery, pH sensitive gels by swelling mechanism, and solubility dependent controlled release systems. For example, Sibalis, U.S. Patent No. 5,013,293 an iontophoretic system that can purportedly

deliver a drug through a patient's skin in pulses. Sibalis apparently accomplishes the pulsatile delivery profile by changing the amount of current applied to the skin, *i.e.*, by changing the magnitude of the driving force transporting the drug through the skin.

Yuk et al., Pharm Res, (1992) 9(7):955-957 describe the electric current-sensitive drug delivery systems using sodium alginate/polyacrylic acid composites. "Pulsatile Drug delivery Current Applications and Future Trends," R. Gurny, H. E. Junginger and N.A. Peppas eds. (May 1993) CRC Press, Inc. describes therapeutic rationale and applications. Creasy et al., Adv "Drug Delivery" Rev (1991) 6:51-56 describes endocrine/reproductive "pulsatile delivery systems. Lippold et al., Acta Pharm Technol, 36(2):97-98 (1990) describes the pulsatile release of potassium chloride from laminated methyl hydroxypropyl cellulose matrixes. Okano et al., "Pulsed and Self-regulated Drug Delivery," Chapter 2, pp. 17-46, (J. Kost ed.) CRC Press, Inc. (1990) describes temperature responsive controlled drug delivery. Nohr et al., Proc Int Symp Controlled Release Bioact Materials, 19th pp. 377-378 (J. Kopecek ed.) describes pulsatile transdermal drug delivery. Yoshida et al., Kagaku Kogaku, 54:919-921 describes the control of pulsatile drug release by thermosensitive poly(N-isopropylacrylamide-co-alkyl methacrylate.

Most prior art pulsatile drug delivery techniques stimulate the drug devices or reservoirs by chemical or physical means to regulate drug release rates from the devices so that a pulsatile type of drug profile can be achieved. This in turn affects the absorption rates and thus produces spikes of drug plasma level. This type of approach is useful in implant devices or in oral dosage forms or in dosage forms that deliver drug to the sites where the drug permeation is not the rate

limiting step. However, this approach does not offer advantage to systems for delivering drug to sites where drug permeation becomes the rate limiting step. An example is transdermal drug delivery.

Berggren and Gale in U.S. Patent No. 4,698,062 attempted to use chemical enhancers to control skin permeation and drug device to control the release rate. In this way they can achieve a pulsatile drug delivery profile. However, this type of system is complicated for manufacturing and is not controllable easily.

Weaver et al. U.S. Patent No. 5,019,034 discloses the use of electroporation in conjunction with a motive force such as iontophoresis to move drugs or other substances into, out of or across tissue. Weaver et al. does not address, however, how to obtain predetermined and/or intermittent delivery of a substance.

An innovative way to achieve pulsatile drug delivery is to maintain the release rate of the drug device substantially constant while altering the membrane permeability at will in a controlled manner. In this way, the drug delivery rate can be altered at any time. Chemical enhancers are not suitable to alter the membrane permeability in an on-demand fashion since their actions are slow and not easily controllable.

Pope et al. U.S. Patent No. 4,723,958 describes a pulsatile drug delivery system in which alternatinv layers of drug and spacer and placed in a tube, and the tube is placed in a fluid environment within the patient. A delivery force is applied to one end of the stack. The drug layers respond to exposure to the fluid to deliver the drug. The spacer layers respond only to the delivery force. The timing of drug pulses depends on the delivery force and the size of the layers, and the duration of a pulse is determined by teh rate of expansion or

dispersion of the active layer into the fluid environment.

There is still a need for methods of sampling or delivery of a substance, such as a biologically active agent, particularly of small molecules to medium macromolecules, like polypeptides, in a controlled or pre-programmed manner. It is also desirable to provide a method to give a faster onset of action in the delivery of pharmaceuticals.

DESCRIPTION OF THE INVENTION

The invention is directed to a method for pulsed transport of a substance through tissue, the method comprising the steps of (a) applying at least one electrical pulse to the tissue to cause electroporation of the tissue region, the electrical pulse being applied for an electroporation pulse duration, the electroporation pulse duration being shorter than the driving force duration; (b) applying a driving force to the region of tissue whereby the driving force causes the substance to be transported through the tissue for a driving force duration; and (c) repeating step (a) during the driving force duration. Specifically, the invention is a method for pulsatile type of delivery of a substance through tissue by using electroporation, combined with either passive diffusion or iontophoresis or both.

In contrast to prior art, the invention uses a single pulse of voltage in the range of about 100 to about 1000 volts and having a duration of about 10 μsec to about 50 msec, followed by similar additional pulses up to about 8 hrs later or even as long as about 7 days. This novel method maintains the permeability of the treated skin high over an extended period of time to permit the transfer of a substance passively by iontophoresis and/or passive diffusion.

Iontophoresis can provide a constant skin flux. After the first pulse, the delivery flux can be increased dramatically (spiked). When the skin permeability reverts to normal after a period of time, the flux will drop to the iontophoretic flux. Subsequent pulsing will give another spike of skin flux. The spikes of delivery rates can be controlled in a pre-determined manner to generate a pulsatile delivery profile for a substance such as a drug.

The method of the invention provides either on demand pulsatile drug delivery/or pre-programmed delivery of a substance such as a drug and faster onset of action. It can also be used to provide a drug delivery spike in response to a measured or detected parameter.

When using iontophoresis as the driving force, the electroporative pulses change the tissue's resistance to iontophoretic transport without causing tissue damage. The magnitude and duration of the electroporative pulse are selected so that the additional current provided to the tissue by the electroporative pulse is less than 0.1% of the total current delivered by both electroporation and iontophoresis over a 30 minute period. The method is therefore a substantially constant current method of providing pulsatile drug delivery or pre-programmed delivery of a substance.

In addition, the method avoids or at least minimizes skin damage caused by high current densities. The magnitude and duration of the electroporative pulse is such that the tissue's resistivity to substance transport can be lowered, and total substance flux increased, without damaging the tissue.

The method of the invention is useful for delivering insulin for patients with diabetes mellitus, antiarrhythmic to patients with heart rhythm disorders, nitrates to patients with angina pectoris, selective

beta-blockade, birth control and general hormone replacement therapy, immunization, cancer chemotherapy, long-term immunosuppression and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of the delivery of fentanyl through human cadaver skin *in vitro*.

Figure 2 is a graph of the results of transdermal iontophoretic delivery of luteinizing hormone-releasing factor (LHRH) using human cadaver skin *in vitro* with or without an electroporative pulse.

Figure 3 is a graph of the results of passive delivery of LHRH using a single application of a 1000 v pulse and a duration of 5 msec.

Figure 4 is a graph of the results of iontophoretic delivery of neurotensin through human skin *in vitro* with or without electroporative pulse.

Figure 5 is a graph of the results of the delivery of salmon calcitonin (sCT).

Figure 6 is a graph of the delivery of sCT.

Figure 7 is a graph of the delivery of LHRH through porcine skin.

Figure 8 is a graph of the results of delivery of molsidomine.

Detailed Description of the Invention

Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is directed. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of this invention, the preferred methods and materials are now described.

All patents and publications cited herein are incorporated herein by reference for the purpose of disclosing and describing information for which the patents and publications are cited in connection with.

While the invention will generally be described with reference to the administering of a drug to a patient, it should be noted that this is a matter of convenience since this administering of a drug to a patient is a preferred embodiment. One of skill in the art will recognize that generally the substitution of a substance for the term drug, the substitution of tissue for patient, and the substitution of transporting a substance out of (sampling/extraction) rather than (delivery) into a patient can be made.

Electroporation as used herein means the use of an electric field to create a temporary decrease in resistance to the transport of substances through a tissue such as a lipid-based barrier or skin. Electroporation is applied *in vivo* (on living tissue) or <u>ex vivo</u> (on excised tissue) or *in vitro* (on artificial tissue). In electroporation, an apparatus comprising a plurality of electrodes, usually of planar steel or other metal such as silver/silver chloride electrodes, disposed on a tissue surface generates an electrical field in the tissue to provide molecular transport of a desired substance from a substance donor reservoir through the tissue into a patient or conversely from a patient through the tissue to a receiver reservoir. The method utilizes means for controlling at least one member selected from the group consisting of the time of initiation of an electroporation pulse, the pulse voltage, the duration of the pulse, the density of the pulse, the interval of time between multiple pulses, the initiation of iontophoresis.

The first electroporative pulse can be initiated any time after application of the electroporation apparatus on the tissue surface, followed by iontophoresis. The subsequent pulsings after the first pulsing can be made at any time after the previous pulsing, continuing up to 24 hours or more. The magnitude of each electro voltage pulsing ranges from about 10 to about 1000 V, with a duration (pulse width) of about 1 μsec to about 50 msec. The iontophorectic current density ranges from 0 (*i.e.*, passive delivery) to about 10 mA/cm^2. Preferably, the invention uses a single pulse of voltage in the range of about 10 to about 1000 V, a duration of about 10 μsec to about 20 msec, with an iontophoretic current density of about 0.05 to about 10 mA/cm^2, followed by similar additional pulses up to about every 8 hours thereafter, especially about every 4 to about every 8 hours or even about every 7 days.

Iontophoresis as used herein means the application of electrical energy to tissue to drive a substance from a reservoir into the tissue. For example, an iontophoretic method and apparatus could use two electrodes placed in contact with the tissue. One of the electrodes is conveniently a pad of absorbent material containing the substance being administered. A voltage is applied between the two electrodes drives the substance from the absorbent material into the tissue. The voltage is preferably in the range of 0.1-50V.

Passive diffusion as used herein refers to the movement of a substance from a reservoir into tissue using a concentration gradient as the driving force.

Transport delivery or sampling/extraction according to the method of the invention is a non-invasive method to transport substances into and through tissue of a subject/patient, including for diagnostic assay, forensic evaluation and drug delivery through

skin. The tissue or dermis can be natural or artificial tissue and can be of plant or animal nature, such as natural or artificial skin, blood vessel tissue, intestinal tissue and the like. The term "artificial" as used herein means an aggregation of cells of monolayer thickness or greater which are grown or cultured *in vivo* or *in vitro* and which functions as a tissue but are not actually derived, or excised, from a pre-existing source or host. The subject/host can be an animal, particularly a mammal, such as dogs, cats, cattle, horses, sheep, rats, mice, and especially is a human being.

Substances that can be transdermally delivered include a wide variety of drugs and diagnostic materials. As used herein, the term "pharmaceutical" or "drug" is broadly defined to include any chemical agent that affects or prevents processes in a living organism. Non-limiting suitable examples of drugs includes those used for therapy, such as antibiotics, drugs intended for prevention, such as vaccines, drugs intended for diagnosis, such as natural and therapeutically introduced metabolites, hormones, enzymes, proteins and the like. Other substances that can be transdermally delivered include enzymes, vitamins, nutrients, DNA, RNA and the like into living organisms. Suitable substances include antiinflammatory drug, analgesics, antiarthritic drugs, antispasmodics, antidepressants, antipsychotic drugs, tranquilizers, antianxiety drug, narcotic antagonists, antiparkinsonism agents, cholinergic agonists, anticancer drugs, immunosuppression agents, antiviral agents, antibiotic agents, appetite suppressants, antiemetics, anticholinergics, antihistaminics, antimigraine agents, coronary, cerebral or peripheral vasodilators, hormonal agents, contraceptive agents, antithrombotic agents, diuretics, antihypertensive agents, cardiovascular drugs, opioids and the like. The substances are capable of

permeating through tissue either inherently or by virtue of the treatment of the skin with the methods of the invention.

Examples of specific drugs include steroids such as estradiol, progesterone, demegestone, promegestone, testosterone, and their esters, nitro-compounds such as nitroglycerine, and isosorbide nitrates, nicotine, chloropheniramine, terfenadine, triprolidine, hydrocortisone, oxicam derivatives such as piroxicam, ketoprofen, mucopolysaccharides such as thiomucase, buprenorphine, fentanyl, fentanyl analogs, naloxone, codeine, dihydroergotamine, pizotiline, salbutamol, terbutaline, protaglandins such as misoprostol and emprostil, omeprazole, imipramine, benzamides such as metoclopramide, scopolamine, peptides such as growth releasing factor and somatostatin, clonidine, dihydroxypyridines such as nifedipine, verapamil, ephedrine, propanolol, metoprolol, spironolactone, thiazides such as hydrochlorothiazide, flunarizine, syndone imines such as molsidomine, sulfated polysaccharides such as heparin fractions and salts of such compounds with physiologically acceptable acids and bases.

The substance can be administered in a physiologically acceptable carrier. Suitable physiologically acceptable carriers are well known in the art and include buffers such as isotonic phosphate buffered saline (PBS), carriers for topical application. and the like.

While not required for use with the transdermal method of the invention, permeability enhancers conventionally known in the art can also be present. Suitable permeability enhancers include fatty acid esters or fatty alcohol ethers of C_{2-4} alkanediols, alcohols

such as ethanol, dimethyl sulfoxide, dimethyl lauramide, polyethylene glycol monolautrate (PEGML) and the like.

The dose and frequency of transdermal administration of a substance by the method of the invention depends on a number of factors, including the drug being used, the intended use, potential skin irritation side effects, the lifetime of the substance, the tissue to which it is administered, the age, weight and sex of any subject or patient. One of skill in the art knows how to evaluate these factors and determine a suitable dose and frequency of administration. A prior art rate control delivery device is designed to release a substance at a rate lower than that obtainable through skin of average permeability and to contain sufficient drug such that unit activity (saturation concentration) is maintained throughout the steady state delivery.

The method can include a step of analyzing a sample obtained from a subject to determine the presence or absence of a substance, the quantity or quality thereof. This can be by the use of specific electrodes or electronic biosensors that utilize a bioactive molecule as the sensing signal-transducing element.

The method can also include the step of automatically administering a drug to the subject in response to a predetermined level of a target substance in the sample or automatically alerting an operator to administer a drug or other treatment in response to a predetermined level of a target substance in the sample, for example orally, dermally, rectally, buccally, intravenously or the like.

EXAMPLES

The following examples are provided to illustrate the invention and should not be regarded as limiting the invention in any way.

Example 1

Flux studies (*in vitro*) were conducted using split thickness human cadaver skin. A piece of skin (0.78 cm^2) separated the donor compartment from the receiver compartment of the diffusion cell assembly. The donor solution contained fentanyl citrate dissolved in isotonic phosphate buffered saline (PBS) at pH 7.4. The receiver solution was PBS at pH 7.4. Fentanyl citrate has a pKa of 7.9. Hence, fentanyl is positively charged at pH 7.4. Therefore, the donor solution contained the anode to ensure electrophoretic mobility. Silver and Ag/AgCl electrodes were used as anodes and cathodes respectively.

The following steps were carried out in four replicates (n=4) in the following sequence:

- thirty minutes of passive delivery
- sixty minutes of iontophoretic delivery at 310 $\mu A/cm^2$ (DC)
- thirty minutes of electroporative pulsing (950 V amplitude, 5 msec pulse width, 1 pulse every 5 sec) superimposed on a current of 310 $\mu A/cm^2$DC. DC was off when pulsing was on.
- ninety minutes of DC iontophoresis (310 $\mu A/cm^2$).
- thirty minutes of electroporative pulsing (950 V, 5 msec pulse width, 1 pulse every 5 sec) superimposed on 310 $\mu A/cm^2$ DC. DC was off when pulsing was on.
- ninety minutes of DC iontophoresis (310 $\mu A/cm^2$)
- passive delivery for 150 minutes

Receiver solution (1 ml) was withdrawn and replaced with an equal volume of PBS at 30 minute

intervals (15 minutes during the pulsing episodes). The withdrawn sample was analyzed for fentanyl content using HPLC.

Figure 1 shows the fentanyl flux as a function of time. The up arrowheads indicate the start and the down arrowheads indicate the cessation of the electrical treatment -- open arrowheads indicate iontophoresis and solid arrowheads represent electroporation.

Between 1.5 and 2 hours, after the 1 hour of iontophoresis has achieved steady state, the initiation of pulsing significantly (> x 2) increases fentanyl flux compared to the flux achieved using iontophoresis. When pulsing is stopped at 2 hours, the flux begins to decline. This trend is more obvious during the second pulsing period -- 3.5 to 4 hours. Pulsing increases the flux and it declines when pulsing is stopped.

The results clearly show that use of electroporative pulsing provides i) programmable drug delivery, and ii) rapid onset of desired flux.

Example 2

Following procedures similar to those described in Example 1 above, the transport of LHRH was determined.

Figure 2 is a graph of the results of transdermal delivery of luteinizing hormone-releasing factor (LHRH) using human cadaver skin *in vitro* with or without a single electroporation pulse. The open squares represent flux with iontophoresis alone and the solid squares represent flux with iontophoresis after pulsing. The solid arrowhead indicates the initiation of pulsing.

Figure 3 is a graph of the results of delivery of LHRH using a single electroporative pulse of a 1000 V pulse and a duration of 5 msec in the absence of iontophoretic current. The solid arrowhead indicates the initiation of pulsing.

Example 3

Following procedures similar to those in the Examples above, the transdermal delivery of neurotensin through human skin *in vitro* was determined.

Figure 4 is a graph of the results of iontophoretic delivery of neurotensin through human skin *in vitro* with or without electroporative pulse. The solid squares represent the electroporative single pulsing at 1000 V and 7 msec. The open squares represent the control without application of electroporative pulsing.

Example 4

Following procedures similar to those described above, the transdermal delivery of salmon calcitonin (sCT) by iontophoresis, iontophoresis and single pulse electroporation and passive delivery was determined. Three replicates (n=3) of cells were used for each method. The sCT donor solution concentration was 0.1 mg/ml in pH 7.4 Dulbecco's PBS and the receiver solution was the same except for the absence of sCT. The tissues were pre-washed with donor buffer for cross-reactivity for two hours.

Figure 5 is a graph of the results of the delivery of sCT. Cells 1-3 (black diamonds) where subjected to iontophoretic treatment at 1 mA/cm^2 (0.385 mA) for two hours, cells 4-6 (black squares) were subjected to a 500V electroporation single pulse and a 1 mA/cm^2 iontophoretic treatment for two hours, and cells 7-9 (open squares) were subjected to passive delivery for 24 hours.

Passive delivery after pre-washing showed a small amount of sCT. The use of electroporation pulse

with iontophoresis increased delivery of sCT over the use of iontophoresis alone.

Example 5

Following procedures similar to those previous Example above, the delivery of sCT was determined. The experimental conditions were the same (1.0 mA/cm^2) except that the pH of the donor and receiver solutions was 4.0 instead of 7.4.

Figure 6 is a graph of the delivery of sCT. The solid squares represent passive delivery only, the open squares represent iontophoresis treatment only and the solid diamonds represent the application of electroporative single pulsing with iontophoresis.

Example 6

Following experimental procedures similar to those described above, transdermal delivery of LHRH was determined. The electroporative conditions to initiate each iontophoresis treatment were 500 V amplitude and 10 msec pulse width.

Figure 7 is a graph of the delivery of LHRH to porcine skin. The solid squares represent iontophoresis treatment alone and the open squares represent the treatment by iontophoresis and single pulse electroporation. The open arrowheads represent when iontophoresis started and the solid arrowheads represent when iontophoresis was discontinued.

Example 7

Following experiments similar to those described above, the electroporative effects on passive permeation of molsidomine through human cadaver epidermis. The donor concentration of molsidomine was 5.0 mg/ml in PBS of pH 7.4 (Dulbecco) and the receiver

solution was PBS of pH 7.4. The electroporative pulsing conditions were 500 V amplitude with a time constant of about 20 msec pulse width.

Results of this experiment are set forth in Figure 8. The open squares represent the control. The solid diamonds represent the treatment with a single electroporative pulse of 500 V amplitude. The passive flux of molsidomine from aqueous pH 7.4 was very low. With a single pulse at the beginning of the experiment, the passive flux was increased over 10 times initially.

WHAT IS CLAIMED IS:

1. A method for pulsed transport of a substance through tissue, the method comprising the steps of:

(a) applying at least one electrical pulse to the tissue to cause electroporation of the tissue region, the electrical pulse being applied for an electroporation pulse duration, the electroporation pulse duration being shorter than the driving force duration;

(b) applying a driving force to the region of tissue whereby the driving force causes the substance to be transported through the tissue for a driving force duration; and

(c) repeating step (a) during the driving force duration.

2. The method of claim 1 wherein steps (a) and (c) are performed according to a preset schedule.

3. The method of claim 1 wherein step (c) is performed on demand.

4. The method of claim 1 wherein step (c) is performed in response to a measured parameter.

5. The method of claim 1 wherein the driving force is selected from the group consisting of passive diffusion, iontophoresis, and both passive diffusion and iontophoresis.

6. The method of claim 5 wherein the driving force is iontophoresis.

7. The method of claim 6 wherein the total current added by the electrical pulse is less than 0.1%

of the total current delivered to the tissue by iontophoresis and the electrical pulse for a driving force duration of 30 minutes.

8. The method of claim 6 wherein in step (a) the pulse magnitude is from about 10V to about 1000V, the duration of the pulse is from about 1 μsec to about 50 msec, and the frequencies of any cycle of iontophoresis is up to about 8 hrs.

9. The method of claim 1 wherein the substance is a pharmaceutical.

10. The method of claim 9 wherein the pharmaceutical is selected from the group consisting of an antibiotics, a vaccine, a metabolite, a hormone, an enzyme, and a protein.

11. The method of claim 9 wherein the pharmaceutical is selected from the group consisting of an antiinflammatory drug, an analgesic, an antiarthritic drug, an antispasmodic agent, an antidepressant, an antipsychotic drug, a tranquilizer, antianxiety drug, narcotic antagonist, an antiparkinsonism agent, an cholinergic agonist, an anticancer drug, an immunosuppression agent, an antiviral agent, an antibiotic agent, an appetite suppressant, an antiemetic, an anticholinergic agent, an antihistaminic agent, an antimigraine agent, a coronary, cerebral or peripheral vasodilator, a hormonal agent, a contraceptive agent, an antithrombotic agent, a diuretic agent, an antihypertensive agent, a cardiovascular drug, and an opioid.

12. The method of claim 9 wherein the pharmaceutical is selected from the group consisting of estradiol, progesterone, demegestone, promegestone, testosterone, and their esters, nitroglycerine, and isosorbide nitrates, nicotine, chloropheniramine, terfenadine, triprolidine, hydrocortisone, oxicam derivatives such as piroxicam, ketoprofen, thiomucase, buprenorphine, fentanyl, fentanyl analogs, naloxone, codeine, dihydroergotamine, pizotiline, salbutamol, terbutaline, misoprostol, emprostil, omeprazole, imipramine, metoclopramide, scopolamine, growth releasing factor, somatostatin, clonidine, nifedipine, verapamil, ephedrine, propanolol, metoprolol, spironolactone, hydrochlorothiazide, flunarizine, molsidomine, heparin fractions and salts of such compounds with physiologically acceptable acids and bases.

13. The method of claim 9 wherein the drug is selected from the group consisting of fentanyl, luteinizing hormone-releasing factor (LHRH), neurotensin, molsidomine and salmon calcitonin (sCT).

14. The method of claim 1 wherein the substance is selected from the group consisting of enzymes, vitamins, nutrients, DNA, and RNA.

15. The method of claim 1 wherein the tissue is skin.

16. The method of claim 1 wherein the tissue is artificial tissue.

17. The method of claim 1 wherein the transport is controlled by a means for controlling at least one member selected from the group consisting of

the time of initiation of the electrical pulse, the electrical pulse voltage, the duration of the electrical pulse, the iontophoretic current density, the interval of time between multiple electrical pulses, the initiation of iontophoresis.

18. The method of claim 1 wherein the electrical pulse in the range of about 10V to about 1000V, with a duration of about 10 μsec to about 20 msec and an iontophoretic current density of about 0.05 to about 10 mA/cm^2, step (c) being performed up to about 8 hrs later.

19. The method of claim 18 wherein step (c) is performed about every 4 to about every 8 hours.

20. The method of claim 1 wherein step (c) is performed up to 24 hours after step (a), the magnitude of the electrical pulse ranging from about 10 to about 1000V with an electroporation pulse duration of about 1.0 μsec to about 50 msec., the iontophoretic current density ranging from about 0 to about 10 mA/cm^2.

21. The method of claim 1 further comprising a step of analyzing a sample obtained from a subject to determine the presence or absence of a substance, the quantity or quality thereof.

22. The method of claim 21 wherein the analyzing is by use of specific electrodes or electronic biosensors that utilize a bioactive molecule as the sensing signal-transducing element.

23. The method of claim 21 further comprising the step of automatically administering a drug to the

subject in response to a predetermined level of a target substance in the sample or automatically alerting an operator to administer a drug or other treatment in response to a predetermined level of a target substance in the sample.

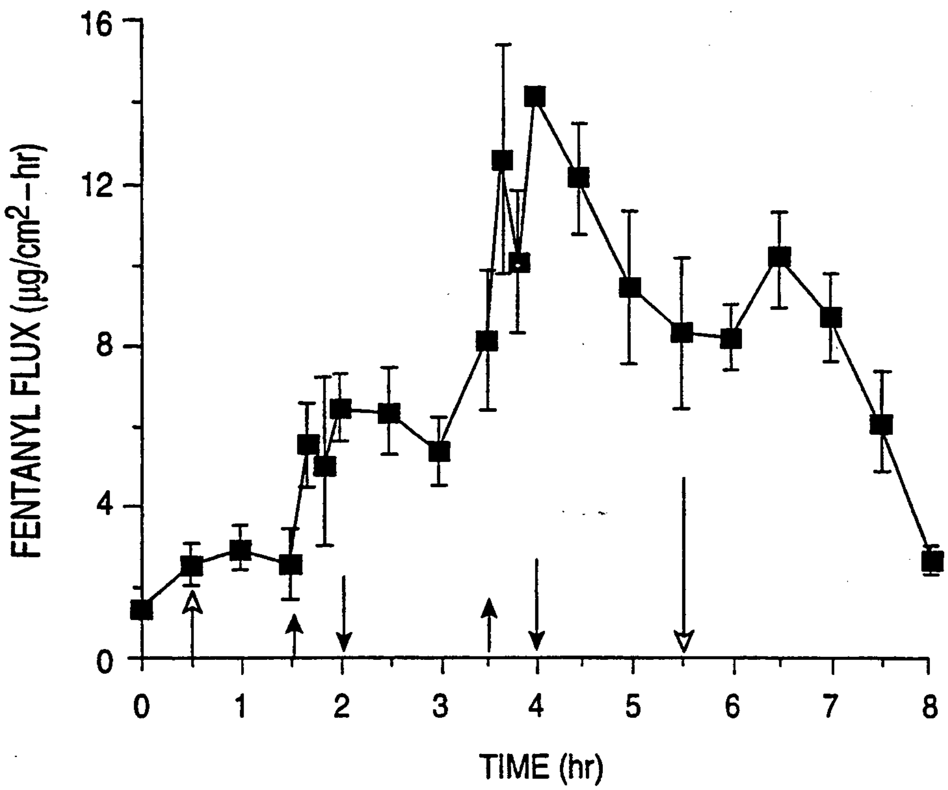

FIG. 1

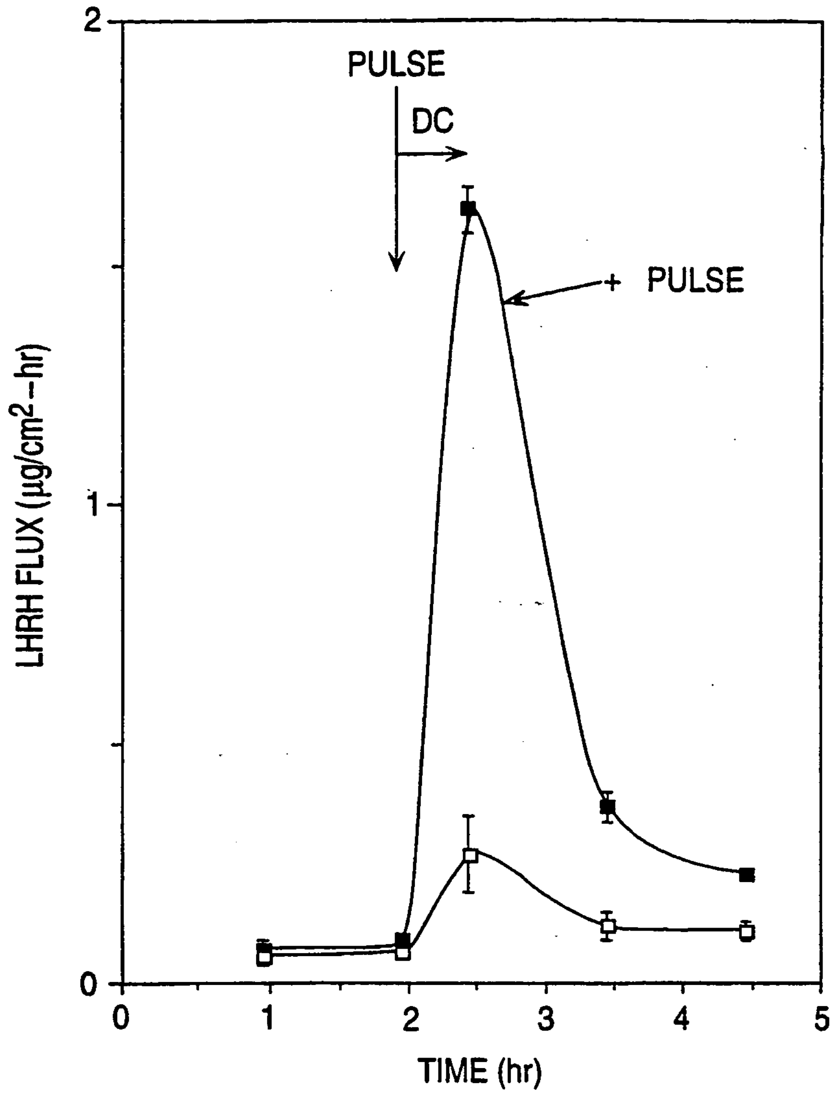

FIG. 2

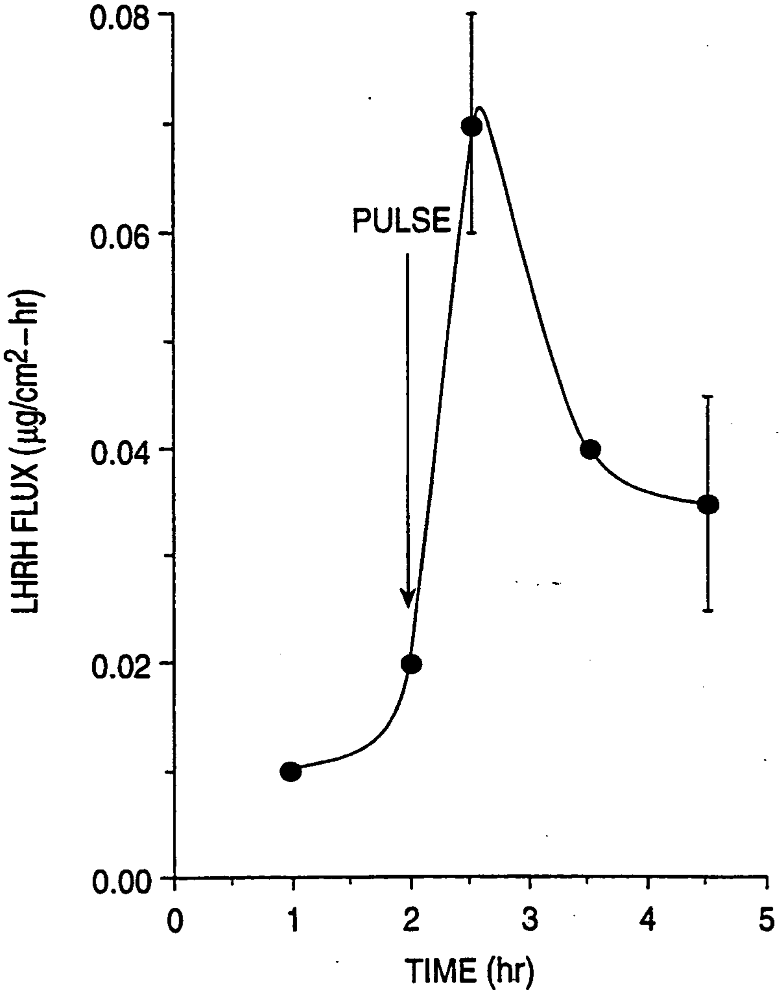

FIG. 3

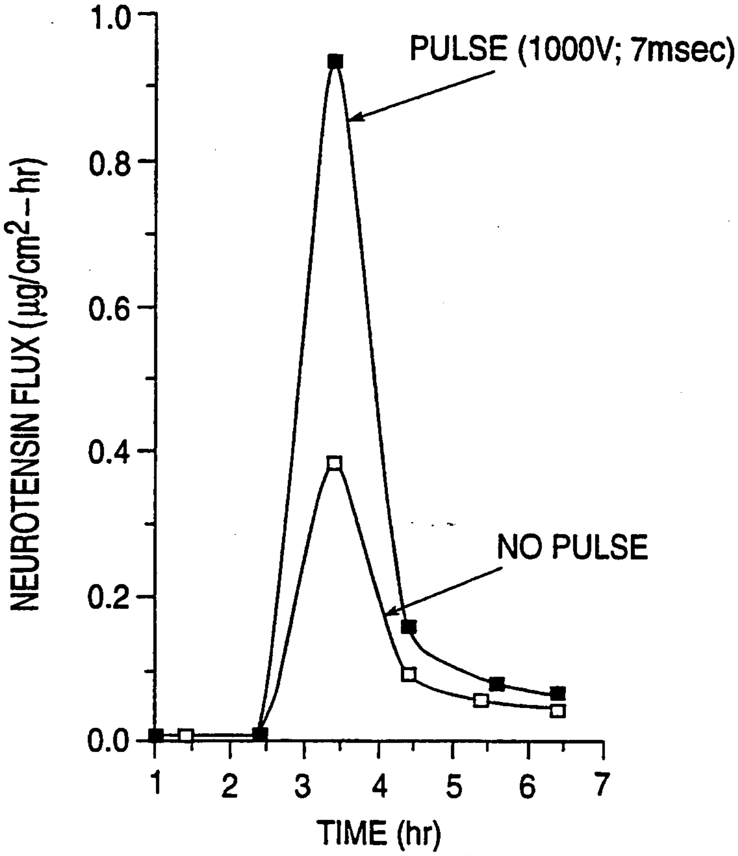
PULSE (1000V; 7msec)
NO PULSE
NEUROTENSIN FLUX (μg/cm2–hr)
TIME (hr)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7

FIG. 4

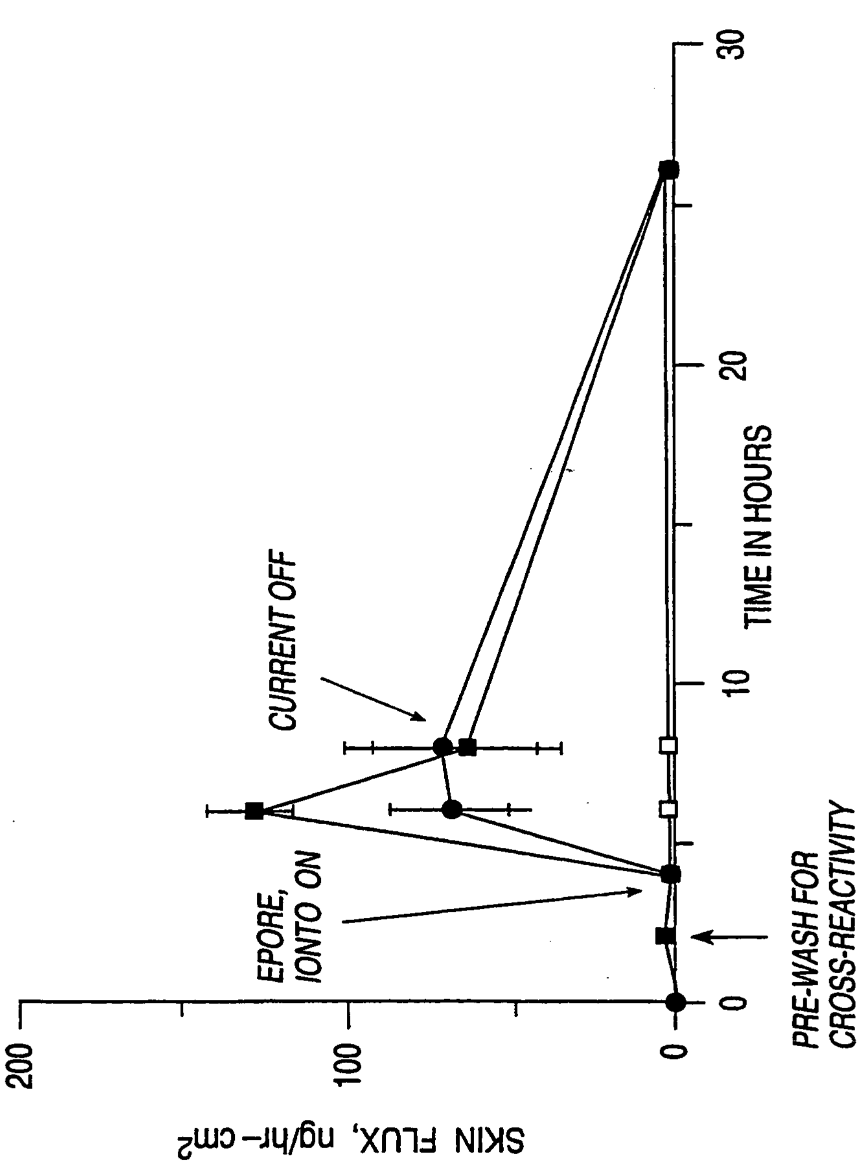
SKIN FLUX, ng/hr-cm2
200
100
0
0
10
20
30
TIME IN HOURS
EPORE, IONTO ON
CURRENT OFF
PRE-WASH FOR CROSS-REACTIVITY

FIG. 5

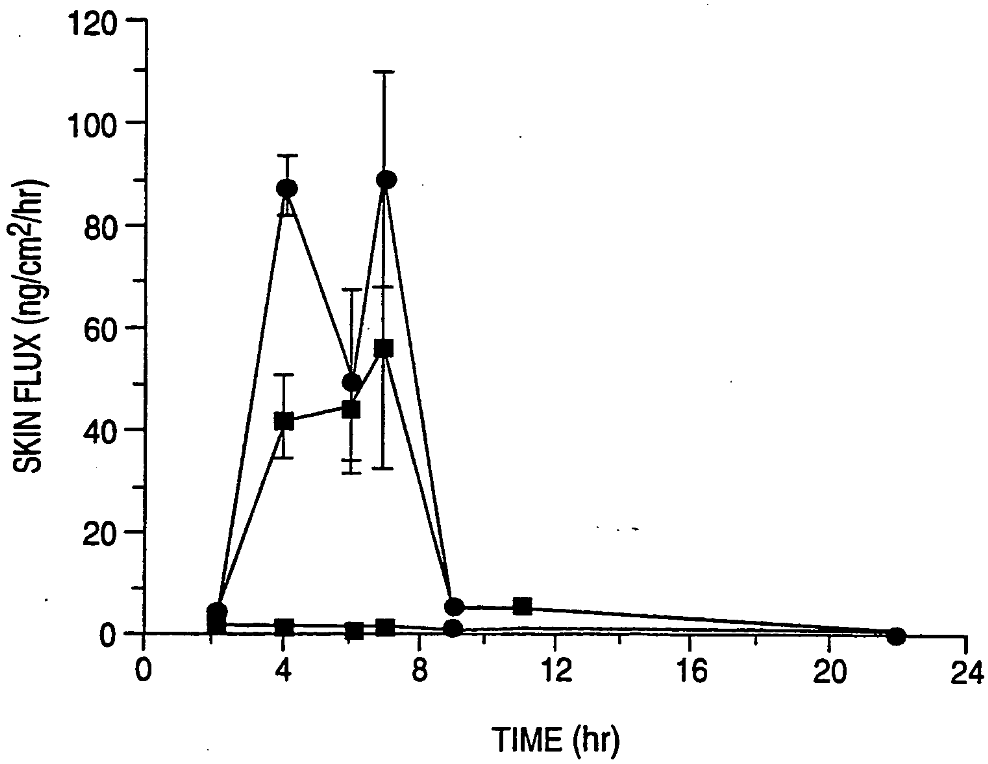

FIG. 6

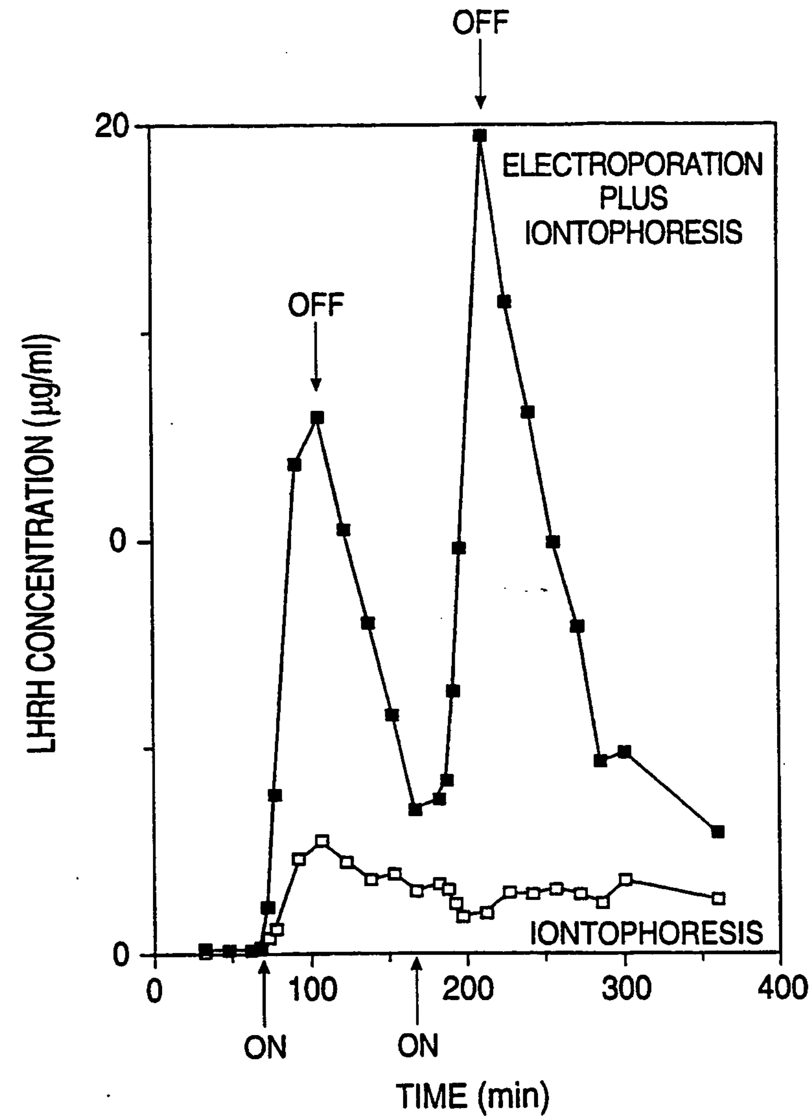
OFF
20
ELECTROPORATION
PLUS
IONTOPHORESIS
OFF
LHRH CONCENTRATION (µg/ml)
0
0
IONTOPHORESIS
0
100
200
300
400
ON
ON
TIME (min)

FIG. 7

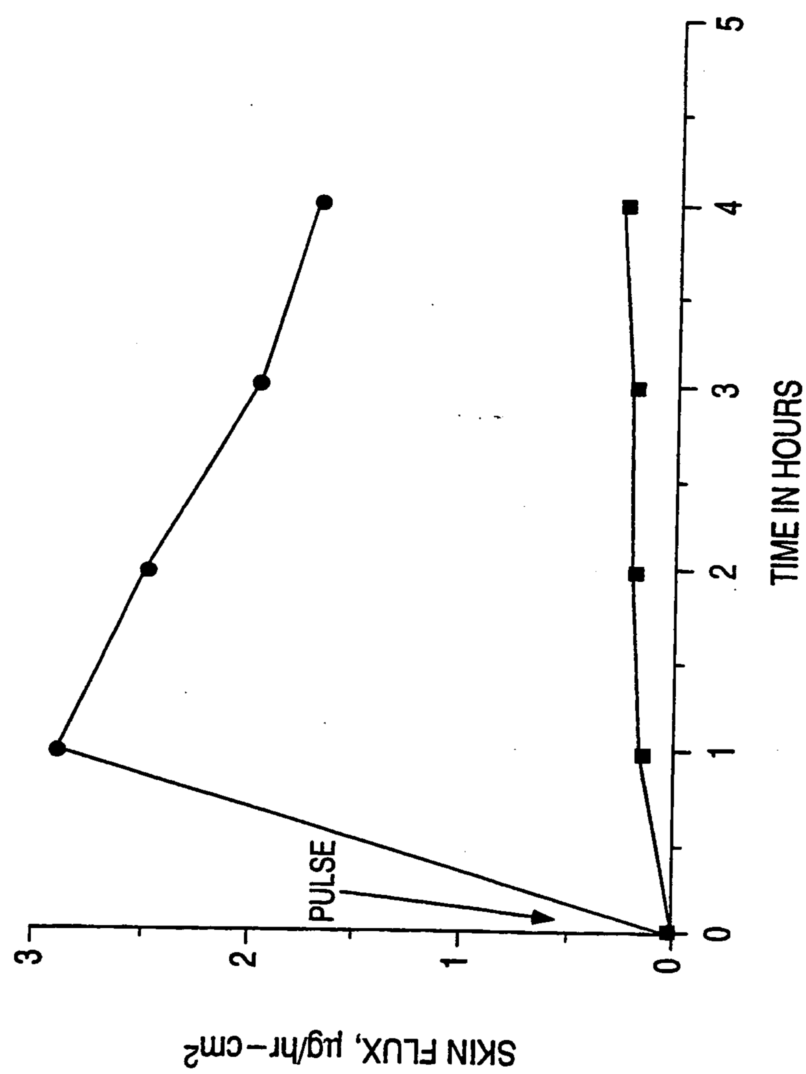

FIG. 8

INTERNATIONAL SEARCH REPORT

Inte[illegible]nal Application No
PCT/US 95/07951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61N1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP,A,0 625 360 (TACHIBANA) 23 November 1994 see abstract see column 9, line 10 - line 28	1,2,5,6
Y	see column 11, line 34 - line 46 ---	9,11,12
Y	WO,A,93 10854 (ALZA CORPORATION) 10 June 1993 see page 2, line 26 - page 4, line 7 see page 2, line 11 - line 25 -----	9,11,12

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 1995	21. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Taccoen, J-F

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 95/07951

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0625360	23-11-94	JP-A-	6070987	15-03-94
		WO-A-	9405368	17-03-94
WO-A-9310854	10-06-93	AU-A-	3235793	28-06-93
		CA-A-	2121372	10-06-93
		EP-A-	0615461	21-09-94
		JP-T-	7501468	16-02-95
		US-A-	5374242	20-12-94
		ZA-A-	9209386	07-06-93